with 1 ug of plasmid which included 139.5 U/mL of Hylenex was then electroporated using the same vacuum cup **2** as in the preceding group; (4) "Vacuum w/o HYA 1 ug" (purple plot)—A 100 uL mantoux injection with 1 ug of plasmid was then electroporated using the same vacuum cup **2** as in groups (2) and (3). FIGS. **29**B and **29**C show the cellular immune responses in terms of spot forming units at Week 2 (FIG. **29**B) and week 4 (FIG. **29**C) of the study in FIG. **29**A.

[0238] Referring now to FIGS. **43**A-**43**C, a 7-day study evaluated the comparative effect of a single, high-volume injection and vacuum-assisted electroporation treatment versus a multi-injection, multi-electroporation treatment using an intradermal needle-electrode electroporation device in guinea pigs. The single high-volume vacuum-assisted electroporation treatment was performed using a 15 mm diameter vacuum cup having a pair of annular ring electrodes and a center (concentric) electrode, similar to the vacuum cup shown in FIG. **15**A. The single injection was a 0.8 mL mantoux injection of plasmid encoding secreted alkaline phosphatase (SEAP) co-formulated with 135 U/mL of hyaluronidase, followed by vacuum-assisted electroporation. The multi-injection, multi-electroporation treatment comprised six (6) individual 0.1 mL mantoux injections each followed by electroporation using intradermal needle electrodes (totaling 0.6 mL of injectate and six (6) applications of electroporation). FIG. **43**A shows the vacuum cup positioned over the injectate prior to application of vacuum pressure. FIG. **43**B shows the injectate within the vacuum cup during application of vacuum pressure, in which is can be seen that the injectate within the tissue has deformed around the center electrode, thereby concentrating the injectate at the electroporation field (see FIG. **40**B). FIG. **43**C shows SEAP expression (as a readout for systemic protein production in the subjects) for both treatments at days 0, 1, 2, 6, and 7. This study demonstrates that the single, high-volume, vacuum-assisted electroporation treatment using the vacuum cup performs substantially equivalent to the six-injection, six-electroporation treatment using the needle-electrode device.

[0239] These studies demonstrate that vacuum-assisted electroporation using the devices and assemblies of the present disclosure enables high-volume delivery of DNA into skin. Furthermore, hyaluronidase formulations (e.g., Hylenex) enhances immunogenicity following vacuum-assisted electroporation of skin. Moreover, the vacuum cups described herein are adapted to take advantage of the significantly higher injectate volumes in intradermal tissue provided by hyaluronidase formulations, including injectate volumes of 1000 uL (1 mL) or higher. Stated differently, by employing hyaluronidase formulations with the vacuum cups of the present disclosure, the vacuum cups can treat significantly larger volumes of intradermal tissue. Additionally, the devices and assemblies of the present disclosure produce more rapid humoral responses than the intradermal needle-electrode electroporation device and comparable overall humoral immune responses relative to the intradermal needle-electrode electroporation device. Furthermore, these studies demonstrate that cellular response kinetics and magnitude can be enhanced through vacuum-assisted electroporation of intradermal tissue. The inventors have also found that using hyaluronidase formulations with vacuum-assisted electroporation of intradermal tissue effectively allows transfection of dermal layers below the superficial layer.

[0240] Referring now to FIG. **44**, an 8-week study compares humoral immune responses data effect of electroporation pulse firing pattern on immunogenicity. In this study, humoral and cellular immune responses in guinea pigs were tested after intradermal treatments of a MERS DNA vaccine via mantoux injection and subsequent vacuum-assisted electroporation using vacuum cups having the electrode arrays shown in FIGS. **40**A-**40**B. FIG. **41**A shows humoral immunogenicity ELISA data at weeks 0, 2, 4, and 8 for both array configurations. Both groups were treated at weeks 0, 2, and 4. FIG. **41**B charts cellular immune response ELISpot data at week 4 and subsequent to week 4 during the same study shown in FIG. **41**A. This study demonstrates that the array configurations performed similarly in terms humoral response, while the concentric array significantly outperformed the opposed array in terms of cellular response.

[0241] Referring now to FIGS. **45**A-**47**C, fluoroscopic images show comparative tissue deflection in guinea pigs during jet injection at various vacuum pressures and nozzle-to-skin offset distances using a jet-injection vacuum cup configured similar to the vacuum cup **902** shown in FIG. **9**. The injectate used in these images is 50% Omnipaque 350 solution to allow radiographic imaging. In each of these images, a superimposed lateral reference line indicates the distal end of the vacuum cup (and thus the distal end of the vacuum chamber and the initial skin-chamber interface prior to vacuum application). FIGS. **45**A-**45**C show jet injection performed without application of vacuum pressure within the chamber. FIGS. **46**A-**46**C show jet injection performed with vacuum pressure applied within the chamber and without a nozzle-to-skin offset distance. FIGS. **47**A-**47**C show jet injection performed with vacuum pressure applied within the chamber and with a nozzle-to-skin offset distance of 3 mm. It should be noted that FIGS. **45**A, **46**A, and **47**A show the tissue pre-injection; FIGS. **45**B, **46**B, and **47**B show the tissue during jet injection; and FIGS. **45**C, **46**C, and **47**C show the tissue post injection.

[0242] As shown in FIGS. **45**A-**45**C, without application of vacuum pressure in the chamber, the jet causes significant tissue deflection (FIG. **45**B), after which the tissue springs back toward the nozzle post-injection (FIG. **45**C), although the injectate resides generally below the vacuum chamber.

[0243] As shown in FIGS. **46**A-**46**C, when vacuum pressure is applied within the chamber during injection (FIG. **46**B), tissue deflection is eliminated. However, as shown in FIG. **46**C, the lack of a nozzle-to-skin offset distance results in the injectate residing below the vacuum chamber post-injection.

[0244] Referring now to FIGS. **47**A-**47**C, when the jet injection is performed with a nozzle-to-skin offset distance of 3 mm and while vacuum pressure is applied within the chamber, tissue deflection is substantially eliminated during injection (FIG. **47**B). When the skin is pulled into the vacuum chamber prior to injection, as in this study, there is intimate contact between the jet nozzle and the skin during injection, and the vacuum pressure is sufficient to prevent tissue deflection. Moreover, post-injection (FIG. **47**C), the injectate resides within the chamber and greater vertical distribution compared to the non-offset setting shown in FIG. **46**C, in which the injectate is compressed into a smaller vertical space. These tests demonstrate significant benefits provided by the jet-injection vacuum cups disclosed herein in terms of injectate fluid distribution.